

Instrument: Pegasus® BTX

Analysis of Musty Odorous Compounds in Drinking Water by SPME GC-TOFMS

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Key Words: Drinking Water, Odor, Environmental, Solid Phase Microextraction, SPME, GC, MS, TOFMS

Introduction

Complaints received by drinking water industries are often related to bad taste and/or unpleasant odors in drinking water. Most complaints involve the presence of chlorine or earthy/musty-smelling compounds. These issues can lead consumers to perceive the water as "unsafe", even though the substances causing the issues are typically not harmful. Reliable detection, identification, and quantification of the analytes behind these unpleasant tastes and odors in drinking water is essential for addressing these problems effectively.

For the past 30 years, it has been widely accepted that musty off-flavors in drinking water are associated with haloanisoles.¹⁻³ Haloanisoles are compounds with a strong musty odor and a very low odor detection threshold. For instance, the odor threshold for 2,4,6-trichloroanisole, which has an aroma described as musty, moldy, or earthy, ranges from 0.05 to 4 ng/L. The formation of these haloanisoles is likely triggered by the microbiological methylation of halophenols during water treatment or transport through the distribution system.⁴⁻⁷ Halophenols, the precursors of haloanisoles, are formed during chlorine disinfection of drinking water and have also been identified as natural halogenation products.^{8,9}

The identification of these compounds in water poses a significant analytical challenge due to their extremely low concentrations, which make them difficult to detect. The most commonly used methods for detecting and identifying odorous compounds in water include Closed Loop Stripping Analysis (CLSA), Solid-Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE),^{10,11} often combined with gas chromatography-mass spectrometry (GC-MS).

"Stripping" techniques, such as CLSA, are not as efficient for less volatile or more polar compounds. To address this limitation, some researchers have used SPME. From a chromatographic perspective, GC-MS remains the preferred analysis method, as it combines separation, identification, and quantification capabilities.

Despite these advancements, the determination of these compounds remains challenging due to their extremely low odor thresholds. Conventional MS or tandem MS/MS techniques can aid in detection, though identification is often limited to a few ions.

The Pegasus BTX GC-TOFMS system offers a solution to this challenge with the acquisition of full mass spectra even at concentrations below 50 pg/L, thanks to its exceptional sensitivity.

The aim of the present study was to analyze and quantify thirteen odorous organic compounds in water using the SPME combined with GC-TOFMS detection. These compounds (Table 1) must be quantified at sub-nanogram/L level in order to be under or close to their Odor Threshold Concentration (OTC).

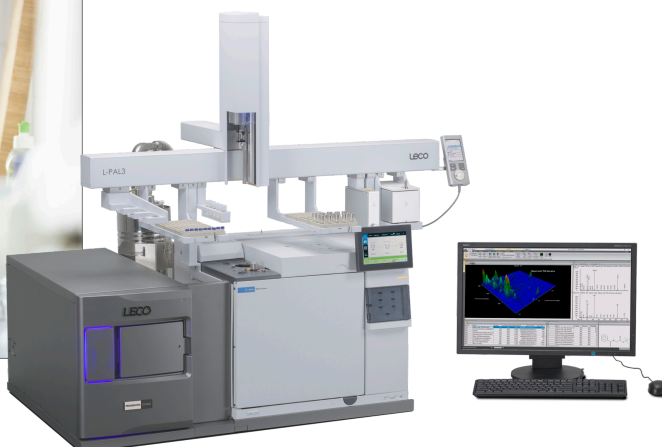
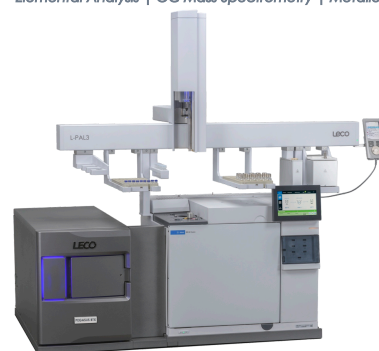


Table 1. Haloanisoles' target list with OTC levels, CAS no., Quantifier m/z and R.T. for the investigated compounds

Name	Acronym	OTC (ng/L)	CAS	Quantifier m/z	R.T. (s)
4-chloroanisole	4-CA	n/a	623-12-1	142.01	666.182
2-chloroanisole	2-CA	5-10	766-51-8	142.01	681.727
4-bromoanisole	4-BA	n/a	104-92-7	185.95	749.084
2,4-dichloroanisole	24-CA	n/a	553-82-2	175.97	753.865
2-bromoanisole	2-BA	n/a	578-57-4	185.95	758.809
2,6-dichloroanisole	26-CA	n/a	1984-65-2	175.97	830.460
2,4,6-trichloroanisole	246-TCA	0.1	87-40-1	211.95	866.157
2,3,6-trichloroanisole	236-TCA	0.1	50375-10-5	211.95	901.855
2-bromo-4-chloroanisole	2-B4A	n/a	60633-25-2	221.91	903.755
2,3,4-trichloroanisole	234-TCA	0.2-2	54135-80-7	211.95	1005.45
2,3,4,6-tetrachloroanisole	2346-TeCA	0.2-2	938-22-7	230.85	1020.65
2,4,6-tribromoanisole	246-TBA	0.05	607-99-8	343.75	1085.04
Pentachloroanisole	PCA	4	1825-21-4	264.82	1156.84

Experimental

Instrumental Parameters

A LECO Pegasus BTX instrument was used for the separation and detection of these substances. The LECO L-PAL3 autosampler, equipped with a SPME conditioning station and agitator, was utilized to automate the fiber conditioning and sample preparation. The SPME fiber was a DVB/C-WR/PDMS with a phase thickness of 80 μm , provided by Restek (Bellefonte, PA, USA). The SPME conditions as well as the GC and TOFMS parameters are summarized in Table 2.

Table 2. SPME GC-TOFMS Method

GC Parameters	Agilent 8890 Gas Chromatograph
Injection (S/SL)	SPME Incubation: 40 °C (1 min), Extraction: 30 min, Desorption: 3 min, Injector temperature and mode: 250 °C, splitless mode
Column 1	Rxi-5 Sil MS 30 m x 0.25 mm x 0.25 μm (Restek)
Carrier Gas	Helium, 1.4 mL/min, constant flow
Oven Program	50 °C (2 min), 10 °C/min to 200 °C, 25 °C/min to 300 °C (2 min)
Transfer Line	280 °C
TOFMS	LECO Pegasus BTX
Ion Source	250 °C
Mass Range	40 – 500 m/z
Acquisition Rate	10 spectra/s

Chemical Standards and Reagents

Methanol (pesticide grade) was obtained by Merck (Darmstadt, Germany). Spring water was used to prepare blanks and standards. Standards of 2-bromoanisole, 4-bromoanisole, 2-chloroanisole, 4-chloroanisole, 2,4-dichloroanisole, 2,6-dichloroanisole, 2-bromo-4-chloroanisole, 2,4,6-trichloroanisole, 2,3,6-trichloroanisole, 2,3,4-trichloroanisole, 2,4,6-tribromoanisole, 2,3,4,6-tetrachloroanisole, and pentachloroanisole were obtained from Restek (Bellefonte, PA, USA) along with 2,4,6-trichloroanisole-d5 from Supelco (Bellefonte, PA, USA).

Working solutions containing haloanisoles at 2 $\mu\text{g/L}$ (Ws1) and 0.2 $\mu\text{g/L}$ (Ws2) were prepared in methanol. The stock solutions were stored at 4°C for less than 1 month. An internal standard solution of 2,4,6-TCA-d5 was prepared in methanol and water at 20 $\mu\text{g/L}$ and was stored at 4°C for less than 1 month.

Sample vials were filled with 15 mL of water and spiked with 40 μL of the internal standard 2,4,6 trichloroanisole d5 at 20 $\mu\text{g/L}$. The calibration levels ranged between 0.07 ng/L and 13.33 ng/L.

Results and Discussion

The ChromaTOF® software facilitated the identification of target compounds through its advanced deconvolution algorithm, which generates high quality, pure mass spectra. This capability enhances similarity scores when compared to commercially available mass spectral databases, such as NIST. Figure 1 illustrates the raw, deconvoluted, and library spectra for 2,4,6-trichloroanisole at a concentration of 0.5 ng/L. The raw caliper spectrum is contaminated with background and noise resulting in a similarity score of 752, but deconvolution provided a cleaner spectrum that matched the library spectrum with an improved similarity score of 893.

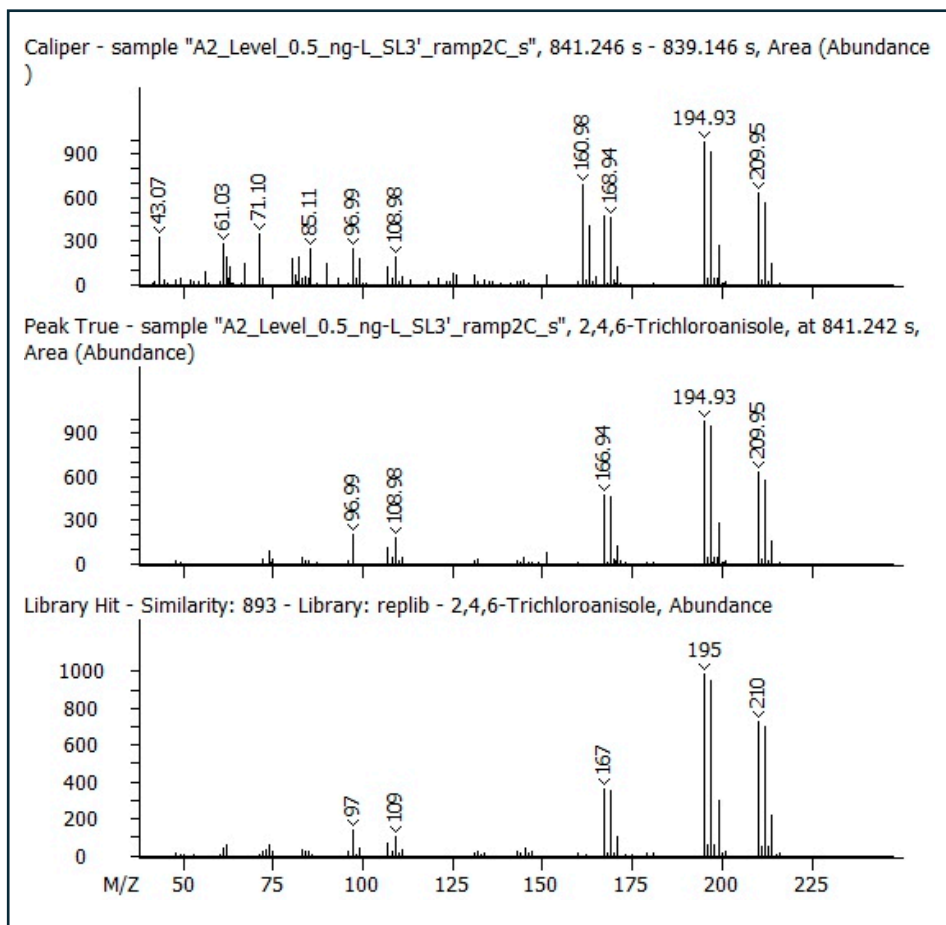


Figure 1. Raw (top), Peak True (after deconvolution, middle) and library spectra (bottom) of 2,4,6-trichloroanisole.

A quant mass was selected from the deconvoluted spectra for each of the target compounds. Two different calibration levels were examined to identify which ions produced the highest signal-to-noise (S/N) ratios, thereby enabling the detection of lower amounts of haloanisoles. The mass accuracy, ranging from 50 to 100 pm, was leveraged to significantly reduce matrix effects and, consequently, minimize background noise around the selected chromatographic peak. This is demonstrated in Figure 2 for 2,4,6-tribromoanisole at a concentration of 0.1 ng/L.

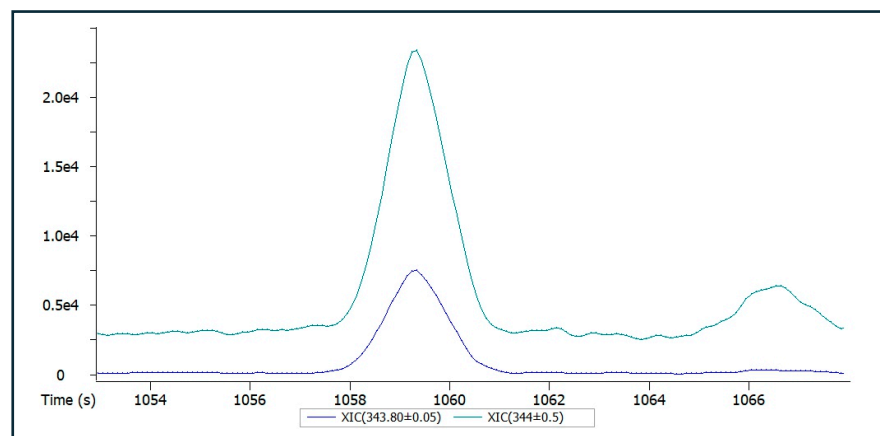


Figure 2. 2,4,6-TBA at 0.1 ng/L chromatographic traces for ions 343.80 m/z (blue) and 344.00 m/z (green).

A chromatogram with all of the target analytes is shown in Figure 3. The chromatogram is generated by plotting the ions providing the best signal-to-noise ratio for each of the target analytes. The separation and detection of the sample is excellent. Additionally, the benefit of using a 3-phase SPME fiber over a PDMS fiber is obvious because the most polar haloanisoles (which would be the least abundant with a 100% PDMS fiber) are the compounds with the most intense response. This SPME technique with the Pegasus BTX provided excellent quantification for all of the compounds considered.

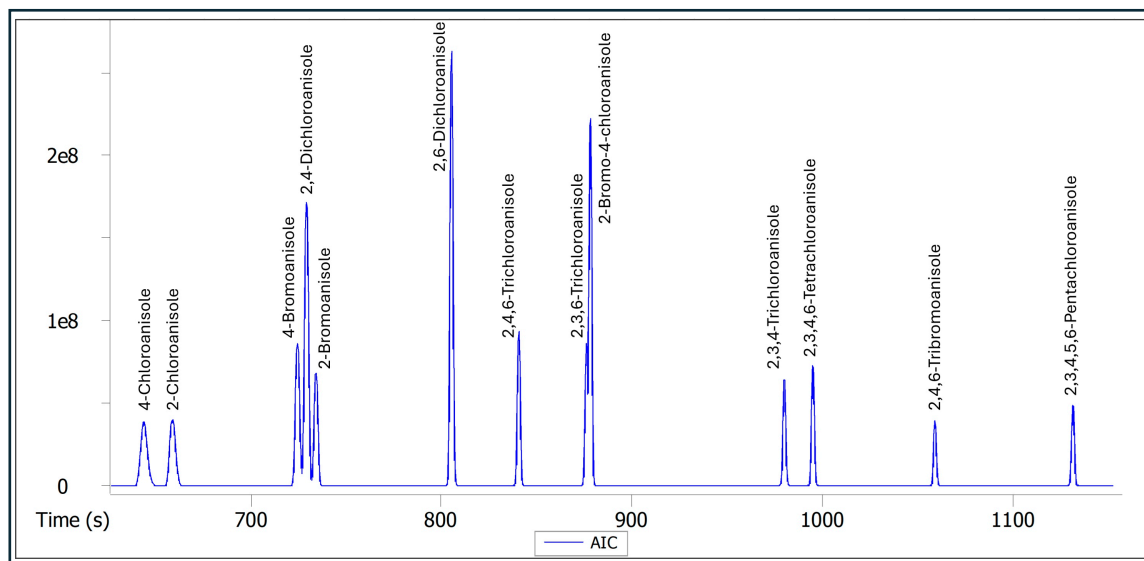


Figure 3. Chromatogram of the haloanisoles standard mixture at 10 ng/L level displaying the ions selected for quantification purposes for each of the compounds.

Calibration linearity for each target was determined as the concentration range where the calibration curve exhibited a linear response, indicated by R^2 values greater than 0.99. The detection limit for this study was defined as the lowest analyte concentration that maintained calibration curve linearity after completing all sample preparation steps. The results are summarized in Table 3, with the calibration curve for 2,4,6-TCA shown in Figure 4.

Table 3. Limit of quantification and R^2 for targeted compounds

Name	LOQ (ng/L)	R^2	Cal. range (ng/L)
4-CA	0.067	0.99915	0.067-13.333
2-CA	0.067	0.99876	0.067-13.333
4-BA	0.067	0.99882	0.067-13.333
24-CA	0.067	0.99907	0.067-13.333
2-BA	0.067	0.99957	0.067-13.333
26-CA	0.067	0.99964	0.067-13.333
246-TCA	0.067	0.99930	0.067-13.333
236-TCA	0.067	0.99928	0.067-13.333
2-B4A	0.067	0.99946	0.067-13.333
234-TCA	0.067	0.99966	0.067-13.333
2346-TeCA	1	0.99106	1-13.333
246-TBA	0.067	0.99954	0.067-13.333
PCA	0.5	0.99992	0.5-13.3331

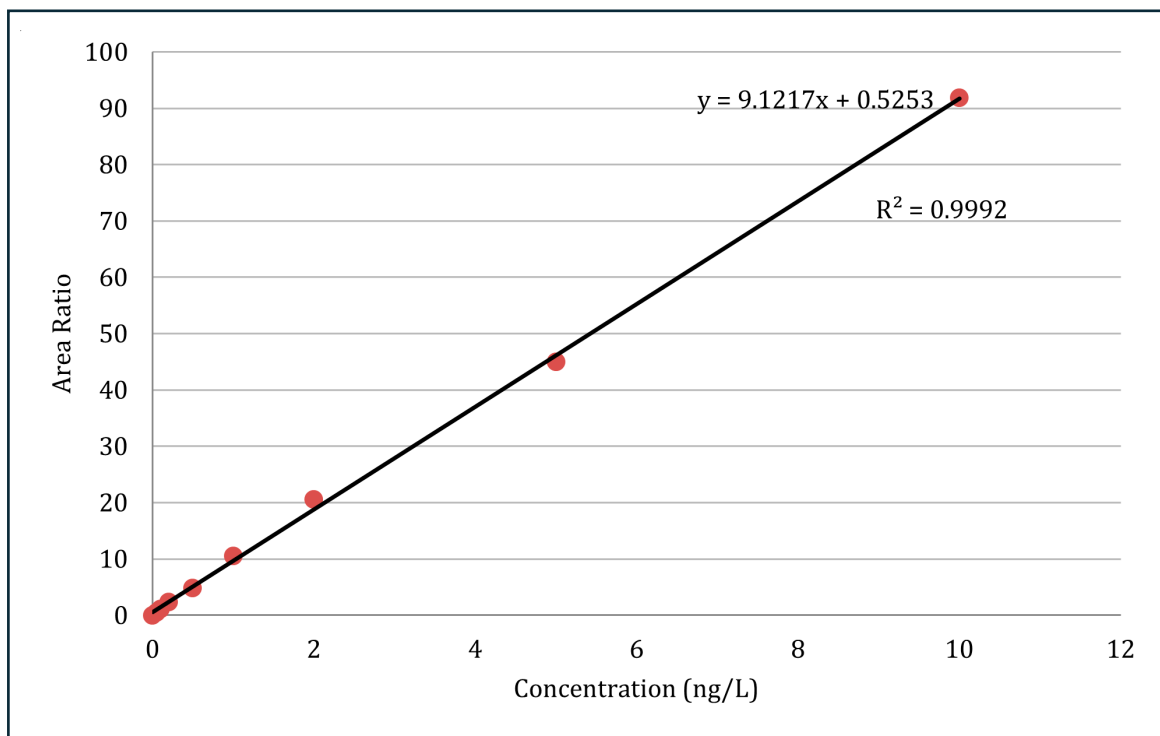


Figure 4. Calibration curve with $R^2=0.9992$ of 2,4,6-TCA.

Table 4 summarizes the calibration information, including the calculated concentration using the calibration curve equations, and the percentage difference between the calculated and certified concentrations.

Table 4. Calculated Concentrations for targeted compounds

Name	Cert. Conc. (lowest) (highest)	Calc. Conc. (lowest) (highest)	% Diff. Conc. (lowest) (highest)
4-CA	0.067	0.066	1.2
	13.333	13.423	0.7
2-CA	0.067	0.072	8.3
	13.333	12.998	2.5
4-BA	0.067	0.056	15.7
	13.333	13.614	2.1
24-DCA	0.067	0.068	2.1
	13.333	13.168	1.2
2-BA	0.067	0.061	9.1
	13.333	13.372	0.3
26-DCA	0.067	0.064	3.6
	13.333	13.351	0.1
246-TCA	0.067	0.072	7.9
	13.333	13.405	0.5
236-TCA	0.067	0.053	21.0
	13.333	13.290	0.3
2-B4CA	0.067	0.076	14.3
	13.333	13.327	0.0
234-TCA	0.067	0.064	3.9
	13.333	13.374	0.3
2346-TeCA	1.000	1.085	7.8
	13.333	12.790	4.1
246-TBA	0.067	0.074	10.4
	13.333	13.383	0.4
PCA	0.500	0.541	7.6
	13.333	13.066	2.0

Regardless of the concentration level or compound considered, the calculated concentration deviated only slightly from the expected concentration, well below 20% different, which is the level required by the French method validation methodology.¹²

Conclusions

The proposed method for analyzing haloanisoles in drinking water offers a promising alternative to traditional approaches. Combining a SPME extraction technique with the Pegasus BTX GC-TOFMS system delivers superior sensitivity, enabling the detection of these compounds at or below their odor threshold concentrations.

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